DIFFERENTIAL ACTION OF CERCORAN AND TOPSIN-M ON SENSITIVE AND TOLERANT STRAINS OF TOXIGENIC FUNGI

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ABSTRACT - The effect of Cercoran and Topsin-M, on physiological activity and mycotoxin production by sensitive strain of *Aspergillus fumigatus* and tolerant strain of *A. flavipes*, was studied in both liquid medium and maize grains. Cercoran and Topsin-M inhibited the mycelial growth and sterigmatocystin production by *A. fumigatus* in liquid medium and maize grains at concentrations varying from 0.5 to 5ppm and 5 to 50 ppm, respectively. Respiration was enhanced at 1 ppm of Cercoran and 1 and 5 ppm of Topsin-M, and inhibited at higher concentrations. Citrinin production by *A. flavipes* was completely inhibited by Topsin-M at 250 ppm in liquid medium and 500 ppm on maize grains. Cercoran at 100 ppm induced 100 % inhibition in citrinin production in both liquid medium and maize grains. Mycelial respiration of *A. flavipes* was significantly promoted with the higher treatments of both Cercoran and Topsin-M. Fatty acids content of maize grains was decreased after 10 days of infection.

RÉSUMÉ - L'effet du fongicide Cercoran et d'un de ses composés, Topsin-M, a été étudié sur l'activité physiologique et la production de mycotoxine par une souche sensible d'*Aspergillus fumigatus* et une souche résistante d'*Aspergillus flavipes*, à la fois en milieu liquide et sur grains de maïs. Dans les 2 conditions de culture, les 2 produits inhibent la croissance mycélienne et la production de stérigmatocystine par *A. fumigatus*, à des concentrations de 0,5 à 5 ppm et 5 à 50 ppm respectivement. La respiration, accrue à 1 ppm de Cercoran et 1 à 5 ppm de Topsin-M, est inhibée aux concentrations plus élevées. La production de citrinine par *A. flavipes* est totalement inhibée par 250 ppm de Topsin-M en milieu liquide et 500 ppm [illegible] grains, et dans les deux conditions par 100 ppm de Cercoran. La respiration d'*A. flavipes* a augmenté de façon significative en présence de concentrations élevées en Cercoran et Topsin-M. Le contenu en acides gras des grains de maïs a diminué après 10 jours d'infection.

KEY WORDS : Cercoran, Topsin-M, sterigmatocystin, citrinin.

INTRODUCTION

Fungal deterioration of stored grains is a common phenomenon reducing both its quality and inducing hence its commercial value. Citrinin occurred as co-contaminants in cereals associated with porcine nephropathy (Krog et al., 1973). Ciegler et al. (1977) and Arai & Hibino (1983) has shown citrinin to be teratogenic in chicken embryos and inducing renal tumors in rats. Sterigmatocystin is reported to be both hepatotoxic and carcinogenic (Lillehoj & Ciegler, 1968; Purchase & Van Der Watt, 1968). Therefore, citrinin and sterigmatocystin are regarded as important mycotoxins which may be ingested by man and animals.

Because of the widespread occurrence of fungi and mycotoxins in agricultural commodities, effective control measures by chemicals or fungicides (which are relatively safe) for post-harvest treatment are still in need. Cercoran is composed of two components, Topsin-M (Tiphanate group) and Thiram (Dithiocarbamate group). Thiophanate group represented a new era in fungicide use when they were introduced in the late 1960's. They are effective at relatively low doses for the inhibition of a broad range of fungi. Topsin-M converted in solution into methyl benzimidazole carbamate (MBC) (Aelbers, 1971; Bollen, 1972) which was toxic 10-fold more than that of parent compound (Vonk & Sijpesteijn, 1971). Ueda & Yoshizawa (1988) found that Topsin-M resulted in the remarkable decrease in contamination levels of trichothecene toxins produced by *F. graminearum* on wheat and barley grains. Dithiocarbamate group represents the most effective fungicide against various mycotoxin-producing fungal isolates (Waginger et al., 1982). Thiram is employed to control fusarial wilt of wheat mould of corn seeds, polysporiosis of flax, ascochitosis of peas, etc.

Experiments were undertaken to determine if, and to what extent, Cercoran and its component Topsin-M affect fungal growth, respiration and mycotoxin production (citrinin and sterigmatocystin) by toxigenic isolates (sensitive and tolerant to benzimidazole), both in liquid medium and on maize grains. Also, they were undertaken to determine the quantitative change in fatty acids content of maize grains.

MATERIALS AND METHODS

Liquid medium:

50ml portion of Czapek-Dox medium were dispensed into each of 250 ml Erlenmeyer conical flasks. The flasks were sterilized at 1.5 atmosphere for 30 min. Fungicides [Topsin-M or Thiphanate-methyl 70 % (1,2-bis(3-methoxy carbonyl-2-thioureido) benzene) and Cercoran or Thiophanate-methyl 50% + Thiram 30% (Bis (dimethyl thio-carbomoyl) disulphide)] were dissolved in ethanol and incorporated to the sterilized medium. The solvent without fungicide was added to the control. Spore suspension of benzimidazole-sensitive strain of *A. fumigatus* and tolerant strain of *A. flavipes* was made separately in sterile distilled water. One ml (approx 10^6 spores) was introduced under aseptic conditions into the flasks. The cultures were incubated at 28°C for 7 days as stationary cultivation. Six flasks of each treatment and control were used for analysis.

1 - Determination of mycelial dry weight

The mycelial of 7 days old cultures were harvested by filtration, washed twice with distilled water, dried over night at 80°C, allowed to cool in a desiccator and then weighed.

2 - Determination of CO_2 evolved

Mycelial respiration was measured by continuous air current method adopted by Kyo Sato (1981). CO_2 evolved from mycelia of treated and untreated fungal species was adsorbed in 0.5 N NaOH solution. Titration was carried out for the unneutralized NaOH with 0.5 N HCl after addition of an excess amount of 3 N $BaCl_2$.

3 - Mycotoxin analysis

a. Extraction procedure

The culture of each treatment was extracted with chloroform, and the extract was concentrated in vacuum. The dry material was transferred to 1-dram vials with small amounts of chloroform, and the solution was evaporated to dryness under a stream of nitrogen.

b. Chemical detection of mycotoxins

Mycotoxins were dissolved in the chloroform and separated by thin-layer chromatography on Silica Gel 60-coated plates using chloroform-methanol (97:3) as solvent for stregmatocystin and ethanol-25% ammonia-water (80:4:16) for citrinin. Sterigmatocystin was determined according to the method of Josefsson & Müller (1977). Citrinin was visualized under long wavelength UV light, scraped off TLC plates and quantified by the colorimetric method of Damodaran et al. (1973).

Maize grains:

25 g of moistened (40% H_2O) autoclaved maize grains in 250 ml Erlenmeyer flask were treated with different concentrations of Topsin-M and Cercoran on the basis of active ingredient. The flasks were inoculated with spore suspension of each *A. fumigatus* and *A. flavipes*. The cultures were incubated at 28°C for 10 days for evaluating citrinin and sterigmatocystin productions and changes in fatty acids content.

1 - Mycotoxins analysis

The samples were extracted with chloroform on rotary shaker for 24 h. The chloroform extract was filtered, concentrated, cleaned up and determined as previously mentioned.

2 - Fatty acid analysis

The infected grains were homogenated in chloroform-methanol (2:1). The suspension was filtered through a G_4 glass filter. Fatty acids (as oleic acid) were determined spectrophotometrically by phosphovanillin method of Zöllner & Kirsch (1962).

Statistical analysis of the results

The least significant difference analysis (L.S.D.) was employed for statistical analysis of the results.

RESULTS AND DISCUSSION

The effect of Cercoran and Topsin-M on mycelial dry weight, respiration and productions of stregmatocystin by *Aspergillus fumigatus* and citrinin by *A. flavipes* in liquid medium was shown in table 1. The growth of *A. fumigatus* was significantly inhibited by Topsin-M at concentrations of 1, 5, 10 and 25 ppm and completely eliminated at 50 ppm. The growth of *A. flavipes* was significantly enhanced at 50 ppm, inhibited at 250 ppm and completely eliminated at 500 ppm.

Rama Devi & Polasa (1984) reported that Bavistin (MBC) at concentration of 25-100 ppm enhanced the growth of *A. versicolor*. At 250 ppm there was an inhibition of growth. MBC interferes with nuclear division of *A. nidulans* (Davidse, 1973, 1976). Also, Bielenin (1987) found that the susceptible isolates of *Pezicula alba* were

Fungicides	Conc. in ppm	*A. fumigatus* Dry wt. mg/100 ml medium	% Inhibition	CO_2 mg/g dry wt.	Sterigmatocystin ug/g dry wt.	% Inhibition	*A. flavipes* Dry wt. mg/100 ml medium	% Inhibition	CO_2 mg/g dry wt.	Citrinin ug/g dry wt.	% Inhibition
	0	780.0	-	56.4	98.0	-	682.0	-	48.4	2040.0	-
Topsin-M	1.0	655.0*	16.0	63.9	116.3*	-	670.0	1.8	43.2	2234.0	-
	5.0	624.0*	20.0	65.8*	0.0*	100.0	663.0	2.8	49.1	2179.0	-
	10.0	461.0*	40.9	32.2*	0.0*	100.0	644.0	5.6	56.5	2151.0	-
	25.0	374.0*	52.0	11.3*	0.0*	100.0	712.0	-	77.7*	1554.0*	23.8
	50.0	0.0*	100.0	-	0.0*	100.0	758.0*	-	96.8*	930.0*	54.4
	250.0	0.0*	100.0	-	0.0*	100.0	398.0*	41.6	145.2*	0.0*	100.0
	500.0	0.0*	100.0	-	0.0*	100.0	0.0*	100.0	-	0.0*	100.0
Cercoran	0.1	804.0	-	63.9	114.0*	-	691.0	-	48.1	2109.0	-
	0.5	714.0*	8.5	40.1	42.9*	56.2	705.0	-	52.0	1818.0	10.9
	1.0	594.0*	23.8	211.1*	26.0*	73.5	730.0*	-	53.2	1653.0*	19.0
	5.0	0.0*	100.0	-	0.0*	100.0	712.0	-	58.7*	1166.0*	42.8
	10.0	0.0*	100.0	-	0.0*	100.0	758.0*	-	92.9*	555.0*	72.8
	50.0	0.0*	100.0	-	0.0*	100.0	226.0*	66.9	233.6*	389.0*	80.9
	100.0	0.0*	100.0	-	0.0*	100.0	0.0*	100.0	-	0.0*	100.0

* Means significant difference compared to the control at 5% level.

Table 1: Effect of Topsin-M and Cercoran on mycelial growth, respiration, sterigmatocystin and citrinin production by *A. fumigatus* and *A. flavipes* in liquid culture.

Tableau I: Effet du Topsin-M et du Cercoran sur la croissance mycélienne, la respiration et la production de sterigmatocystine et de citrine par *A. fumigatus* et *A. flavipes* en culture liquide.

completely inhibited at 0.1 ppm of Benomyl, but the resistant isolate was not affected at 1000 ppm of Benomyl and Bavistin.

CO_2 evolution by mycelia of the sensitive isolate of *A. fumigatus* exhibited two significant responses of activation at 5 ppm and inhibition at 10 and 25 ppm Topsin-M. The respiration of the tolerant isolate of *A. flavipes* was increased at 25, 50 and 250 ppm. Stimulation of respiration has been observed by Naguib et al. (1982). They demonstrated that the low gain in dry weight by patoran treatment was coupled with remarkable increase in respiration. Naguib (1969) found that sevin inhibited respiration and mycelial growth of *R. solani*. Buchanan et al. (1987) recorded that Miconazole inhibited respiration and altered mitochondrial ultrastructure.

Sterigmatocystin production by *A. fumigatus* was enhanced at 1 ppm, but 100% inhibited at 5 ppm Topsin-M. Citrinin production by *A. flavipes* was inhibited by 23.8, 54.5 and 100% at concentrations of 50, 250 and 500 ppm, respectively. Rama Devi & Polasa (1984) noted that Bavistin at lower concentrations stimulated aflatoxin production. At 250 ppm, Bavistin inhibited stregmatocystin production in synthetic liquid medium. Sevin, Carbofuran and Metalkamate (Carbamate insecticides) at 100 ppm, inhibited aflatoxin production by 55, 47 and 97% respectively, in YES broth (Draughton et al., 1983).

Cerconan (Topsin-M + Thiram) was toxic 10-fold more than Topsin-M (table 1). It inhibited the mycelial growth of *A. fumigatus* at 0.5, 1 and 5 ppm. The mycelial growth of *A. flavipes* was enhanced at concentrations of 0.1 to 10 ppm, but inhibited by 66.9 % at 50 ppm. Stimulation of growth has been observed in *A. flavus* IMI 89717 cultures treated with 10 and 100 ppm of Rizolex-Thiram (Hasan, 1988). Waginger et al. (1982) found that a mixture of different Dithiocarbamate fungicides proved to be most effective against various mycotoxin-producing fungal isolates.

Respiration was significantly promoted in mycelia of both aspergilli, at different concentrations of Cercoran, 0.1 and 1 ppm in *A. fumigatus* and 5, 10 and 50 ppm in *A. flavipes*. Respiration was stimulated by Linuron at 5 to 20 ppm in *P. funiculosum* (Bakalivinov, 1972), by Afugan at 18 ppm in *P. chrysogenum* and by Brominal at 32.4 ppm in *A. niger* (Omer, 1991).

Sterigmatocystin and citrinin productions were increased at lower concentrations of Cercoran treatment. At higher levels, sterigmatocystin was inhibited by 73.5% at 1 ppm and citrinin was inhibited by 72.8% at 10 ppm. Sharma & Padwal Desai (1989) stated that Benomyl and Carbendazim stimulated biosynthesis of aflatoxins at 0.8 ppm, while they were inhibited at higher concentrations. It is assumed that Benzimidazoles, near the minimal inhibitory concentration, reduce the activity of tricarboxylic acid cycle. This may lead to an accumulation of acetyl coenzyme A, which is an essential intermediate in the biosynthesis of aflatoxin. Hasan (1988) found that Rizolex-Thiram inhibited production of citrinin by *P. chrysogenum* and *P. corylophilum* at 100 ppm and aflatoxin by *A. flavus* IMI 89717 at 25 ppm.

The effect of Topsin-M and Cercoran on production of sterigmatocystin by *A. fumigatus* and citrinin by *A. flavipes* on maize grains, in addition to the changes in fatty acids content of grains were shown in table 2. Sterigmatocystin was inhibited by 65.5% at 5 ppm of Topsin-M and by 70.9% at 1 ppm of Cercoran. Citrinin was inhibited at 250 ppm of Topsin-M by 69.6% and by Cercoran at 50 ppm by 54.2%.

Inhibition of mycotoxin production had been previously discussed by Rama Devi & Polasa (1984). They concluded that Carbendazim inhibited sterigmatocystin production by *A. versicolor* on maize grains and its flour at 500 ppm. Draughton & Churchville (1985) reported that Maneb (Dithiocarbamate), Carbofuran, Metalkamate

Fungicides	Conc. in ppm	*A. fumigatus*			*A. flavipes*		
		Sterigmatocystin		Fatty acid	Citrinin		Fatty acid
		ug/50g seed	% Inhibition	mg/g seed	ug/50g seed	% Inhibition	mg/g seed
	0	23.0	-	32.4	694.0	-	29.9
Topsin-M	1.0	23.8	-	30.8	722.0	-	27.0
	5.0	7.7*	66.5	37.4*	736.0*	-	25.5
	10.0	5.4*	76.5	39.2*	666.0	4.0	26.9
	25.0	3.8*	83.5	40.1*	583.0*	16.0	27.8
	50.0	0.0	100.0	42.0*	416.0*	40.0	29.4
	250.0	0.0	100.0	44.0*	211.0*	69.6	34.4*
	500.0	0.0	100.0	44.0*	0.0*	100.0	44.0*
Cercoran	0.1	24.5	-	34.2	664.0	4.3	27.1
	0.5	23.0	-	35.2	672.0	3.2	30.4
	1.0	6.7*	70.9	39.3*	708.0	-	34.2
	5.0	0.0*	100.0	43.4*	597.0*	14.0	38.9*
	10.0	0.0*	100.0	44.0*	541.0*	22.0	41.0*
	50.0	0.0*	100.0	44.0*	318.0*	54.2	43.1*
	100.0	0.0*	100.0	44.0*	0.0	100.0	44.0*

* Means significant difference compared to the control at 5% level.

Table 2: Effect of Topsin-M and Cercoran on mycotoxin production and fatty acids content by the toxigenic aspergilli on maize grains.

Tableau 2: Effet du Topsin-M et du Cercoran sur la production de mycotoxines et le contenu en acides gras par les *Aspergillus* toxiques sur grains de maïs.

and Sevin (Carbamate) inhibited zearalenone by *F. roseum* var. *graminearum* in corn kernels by 100, 97, 77 and 94%, respectively. Also, Ueda & Yoshizawa (1988) found that Topsin-M resulted in the remarkable decrease in trichothecene levels produced by *F. graminearum* on wheat and barley grains.

Utilization and degradation of fatty acids by each of *A. fumigatus* and *A. flavipes* on maize grains was suppressed at different concentrations of Topsin-M and Cercoran. Treatment of banana fruits with Bavistin at a concentration of 2000 ppm prevented fruit decay and mycotoxin production by various fungi (Reddy & Gourishankar, 1983; Ved Ram & Dharm Vir, 1984).

CONCLUSION

Ten-fold more toxicity of Cercoran, compared to Topsin-M, is due to Thiram. This result agrees with the finding of Lo (1978). He reported that Homai (Cercoran) gave a better control of seed-borne organisms than Topsin-M. *A. fumigatus* was sensitive 10-fold more than *A. flavipes* to both fungicides. Martin et al. (1984) have reported that *Rhizoctonia solani* and binucleate *Rhizoctonia*- like fungi were sensitive to Benomyl (ED_{50} 10 ppm), whereas isolates of *P. zeae* were tolerant of Benomyl (ED_{50} ppm). Gessler et al. (1980) concluded that adsorption of MBC in sub-lethal rates was higher in mycelium of MBC-sensitive strains of *Botrytis cinerea* and *F. lycopersici* than in resistant ones.

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